Table II. Percentage of larval attachment to rabbits of 100 diapausing larvae immersed at indicated ages (days) in hormonal solutions

Concentration (%)	Larval attachment (%) at day				
	18	28	28	18	42
	α-ecdysone				
0.1	6	10			
1.0		38	36	32	40
	β-ecdysone				
0.1	19	24	28		
1.0	10	38	42	40	46
	trans, trans-10,11-epoxyfarnesenic acid methyl ester[a]				
0.1	0	0	0	0	0
1.0	0	0	0	0	0

[a] Methyl-10,11-epoxy-3,7,11-trimethyl-2,6-dodecadienoic acid. The percentage of attachment indicates the ratio of the number of larvae which attached to the total number present and alive at the end of 48 h.

topically to each larva with a microapplicator. Larvae were kept for 2 to 3 h at 5 °C after hormonal treatment before they were returned to the temperature at which they were maintained normally. One day later, the larvae were placed inside small plastic containers[8] attached to closely clipped rabbits. The percentage of attached (and feeding) larvae was determined at the end of 48 h. Other samples of 100 diapausing larvae were immersed in one of the same solutions for about 15 sec and handled in the same way as those with topically applied solution. Untreated controls from both short and long day photoperiods were placed on rabbits at the same time as the treated larvae. In other tests, the effects of the solvents on mortality and attachment were negligible.

Results recorded in Tables I and II indicate that treatment with either concentration of ecdysones terminated larval diapause in *R. sanguineus*. Treatment of the larvae with both α- and β-ecdysones produced positive results, when applied topically or by immersion in hormone solutions. However, the effects of β-ecdysone were slightly more spectacular than those of α-ecdysone in both ways of treatment. The analogue of juvenile hormone (*trans, trans*-10, 11-epoxy-farnesenic acid methyl ester) was ineffective topically.

It is well established that growth and metamorphosis in insects is regulated by the endocrine activity and the hormones involved such as juvenile hormones[9] and ecdysones[10] have been isolated and synthesized. Furthermore, juvenile hormone and its analogues terminate photoperiodically induced diapause in insects[2–4]. That diapause can also be terminated by ecdysones in larvae of the tick *Dermacentor alpictus* as demonstrated by WRIGHT[11]. Our results, besides substantiating the above author's observations in *R. sanguineus*, suggest that the endocrine mechanisms of the Acarina may be similar to those of the Insecta and Crustacea[12].

Zusammenfassung. Die Diapause von Larven der Zecke *Rhipicephalus sanguineus* (Lat.) wird durch α- and β-Ecdyson beendet.

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[9] A. S. MEYER, H. A. SCHNEIDERMAN, E. HANZMAN and J. H. KO, Proc. natn. Acad. Sci. USA *60*, 8531 (1968).
[10] J. N. KAPLINS, M. J. THOMPSON, R. T. YAMOTO, W. E. ROBBINS and S. J. LOULOUDES, Steroids *8*, 605 (1969).
[11] J. E. WRIGHT, Science *163*, 390 (1969).
[12] V. B. WIGGIESWORTH, Q. Jl. microsc. Sci. *77*, 191 (1943).
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Calcium Dependence of Protein Transport by the Small Intestine of the New-Born Pig

The stimulus to pinocytose is normally given to a cell by the binding of cations to negatively charged groups on the membrane surface. The response to this stimulus is, in amoeba, first a change in membrane resistance[1], then a physical movement of membrane substance to the cell interior. Pinocytosis induced with monovalent cations can be inhibited by high concentrations of calcium. In this case competition between calcium and sodium for anionic sites is thought to remove the stimulus to pinocytose[2]. Usually however the inducing cation is believed to displace calcium as it binds to the membrane to cause the pronounced fall in resistance recorded immediately before vesicle formation begins[1]. Removal of calcium with EDTA also causes the membrane resistance to fall but this does not, by itself, initiate membrane movement[3]. It has therefore been suggested that it is the presence of free calcium which links stimulus to response, possibly by activating a cytoplasmic contractile system analogous to the excitation-coupling seen in muscle[4]. If this were true the action of calcium on a pinocytotic cell would be seen to be complex, high concentrations of the ion inhibiting the stimulus to pinocytose but some calcium being essential for the induction of membrane instability. It seemed therefore of interest to test for calcium interactions in another tissue

[1] P. W. BRANDT and A. R. FREEMAN, Science *155*, 582 (1967).
[2] B. A. COOPER, C. r. Trav. Lab. Carlsberg *36*, 385 (1968).

noted for its pinocytotic activity, the small intestine of the new-born pig.

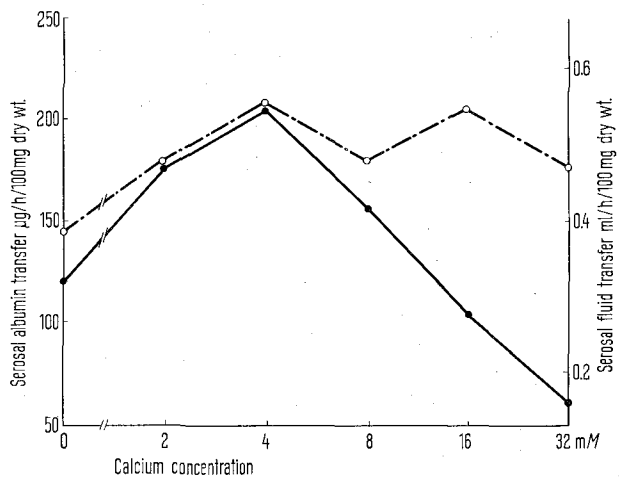
The small intestines of unsuckled new-born piglets were formed into everted sacs as described previously[5]. These were then incubated in a modified saline solution, containing 4% w/v bovine serum albumin and different concentrations of $CaCl_2$. The saline contained 95 m*M* NaCl, 5,7 m*M* KCl, 25 m*M* $NaHCO_3$, 1.2 m*M* $MgSO_4$, 5.5 m*M* glucose and 25 m*M* *tris* buffer pH 7.6. The highest concentration of calcium used (32 m*M*) increased the osmolality of the saline to 370 mosmoles/kg water. Mannitol was added to solutions containing lower concentrations of calcium to maintain this high osmolality in all solutions used. A known volume of saline containing 2 m*M* $CaCl_2$ was placed on the serosal side of each sac at the start of incubation. Fluid transfer was measured at the end of incubation and the amount of albumin transported determined by immunodiffusion[6] using serum taken from rabbits previously injected with bovine serum albumin. Sacs were dried to constant weight at 105 °C and transfers expressed per 100 mg dry weight of tissue.

The dependence of albumin transfer on the concentration of calcium in the mucosal medium is shown in the Figure. Transfer in the absence of calcium, 143 ± 25.7 µg/100 mg/h, increased to a maximum of 211 ± 35.3 µg/100 mg/h at an external calcium concentration of 4 m*M*. Further increases in calcium concentration caused a steady fall in the amount of protein transported. Transfer of albumin from a 32 m*M* $CaCl_2$ saline solution, 70 ± 12.7 µg/100 mg/h, was only $^1/_3$ the value found with 4 m*M* $CaCl_2$. All values give means ± S.E. of 14 observations. These differences were statistically significant (comparison of 4 m*M* versus 32 m*M* $CaCl_2$, $P < 0.001$; comparison of no calcium versus 4 m*M* $CaCl_2$, $P < 0.05$), as was the inhibition of albumin transport caused by increasing the external concentration of calcium from 4 to 16 m*M* ($P < 0.01$). In contrast, the transfer of fluid remained little affected by high calcium concentrations. Fluid transfer increased from 0.44 ± 0.04 to 0.55 ± 0.03 ml/100 mg/h when the calcium concentration was raised from 0 to 4 m*M* ($P < 0.01$), but the transfer in 32 m*M* $CaCl_2$ saline, 0.47 ± 0.06 ml/100 mg/h, was not different from that found using 4 m*M* $CaCl_2$ saline ($P > 0.1$). Thus the rise in albumin transfer, seen on increasing the calcium concentration from 0 to 4 m*M*, could be associated with the increased transfer of fluid, but the fall in albumin transfer seen at higher calcium concentrations, could not.

In a second series of experiments fluid transfer was measured in the presence and absence of albumin at 3 different calcium concentrations. The results are shown in the Table. In this case fluid transfer in the presence of albumin was similar at all 3 calcium concentrations used. Transfer was stimulated by the presence of albumin both in the absence of calcium and with 4 m*M* $CaCl_2$ present. Albumin-dependent fluid transfer could not however be detected using saline containing 32 m*M* $CaCl_2$.

The piglet small intestine exhibits intense pinocytotic activity immediately after birth[7] at a time when it is obtaining a passive immunity to disease through the absorption of antibodies present in the sow's colostrum. Everted sacs of pig intestine, formed at this time, show an enhanced fluid and ion transport in the presence of protein[5]. We take this to indicate that something approaching the normal operation of pinocytosis can take place under in vitro conditions. Protein can no longer stimulate fluid transfer at high calcium concentrations. Albumin transfer is itself inhibited and it appears that pinocytosis is blocked, a situation analogous to that found with amoeba, where calcium inhibits the stimulus to pinocytose[2]. The absence of calcium does not remove the ability of albumin to stimulate fluid transfer. It should not have affected the stimulus to pinocytose but one might have expected the response to be diminished if calcium were required for vesicle formation. The transfer of albumin was however significantly less than that found with 4 m*M* $CaCl_2$ and one might therefore assume a need for calcium to produce an optimal pinocytotic response.

The similarities which exist between amoeba and the intestinal epithelium in their response to calcium during pinocytosis might be important in exposing what is not essential for pinocytosis to take place. Their plasma membranes differ markedly both in electrical properties and in their permeability to sodium ions[3,8], yet both pinocytose when placed in contact with suitable inducers. It is tempting to speculate that it is calcium that controls pinocytosis in both cases either by modifying the stimulus



Calcium dependence of albumin and fluid transport by the new-born pig intestine. Everted sacs were prepared and incubated for 90 min at 37°C in saline containing different concentrations of $CaCl_2$ as described in the text. ○---○, serosal transfer of fluid; ●—●, serosal transfer of bovine serum albumin. Each point gives the mean of 14 determinations.

Effect of calcium on the albumin-dependent fluid transport by new-born pig intestine

Calcium Concentration (m*M*)	Fluid transfer (ml/100 mg/h) Control	Albumin	*P*
0	0.38±0.07 (6)	0.54±0.07 (6)	<0.05
4	0.37±0.05 (10)	0.46±0.06 (10)	<0.02
32	0.47±0.06 (8)	0.48±0.08 (8)	NS

Everted sacs were prepared and incubated in the presence and absence of 4% w/v bovine serum albumin, as described in the text. Numbers give the means±S.E. (No. of observations). *P* gives the significance of the albumin-dependent difference in fluid transfer assessed using a paired *t*-test.

[3] D. L. BRUCE and J. M. MARSHALL JR., J. gen. Physiol. *49*, 151 (1965).
[4] J. M. MARSHALL and V. T. NACHMIAS, J. Histochem. Cytochem. *13*, 92 (1965).
[5] M. W. SMITH, J. Physiol., Lond. *214*, 349 (1971).
[6] P. G. H. GELL, J. clin. Path. *10*, 67 (1957).
[7] A. G. M. MATTISSON and B. W. KARLSSON, Ark. Zool. *18*, 575 (1967).
[8] R. C. ROSE and S. G. SCHULTZ, J. gen. Physiol. *57*, 639 (1971).

to pinocytose or by taking part in a common response to stimuli initiated by other inducers.

Resumen. El transporte de albumina en el intestino delgado del cerdo esta asociado con un aumento en el transporte de fluido. Ambos transportes son inhibidos por altas concentraciones de calcio. Ausencia de calcio tambien inhibe el transporte de albumina. Condiciones optimas en el transporte de albumina y fluido se obtienen usando Cl_2Ca 4 m*M* en el medio. Se deducen analogias al comparar estos resultados con resultados similares en ameba, obtenidos de la literatura.

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High Electrical Discharge Frequency During Aggressive Behaviour in a Mormyrid Fish, *Gnathonemus petersii*

In the mormyrid fish *Gnathonemus* the rate of the electric organ discharge is variable[1–5]. As a large number of investigations indicate, following different kinds of stimulation[6–8] and even during periods of *aggressive* behaviour[4, 9, 10], maximal frequency never exceeded 50–60 Hz. The only observation where mormyrids emitted higher frequencies was made by Lissmann[1], who mentioned for a mechanically stimulated *Gnathonemus senegalensis* emission rates of 130 Hz. The present paper furnishes evidence that *Gnathonemus petersii* emits continuously 'high frequency' bursts of up to 140 Hz during aggressive behaviour. This frequency surpasses the frequency range observed in resting and swimming fish.

Eight *Gnathonemus petersii* (16–12.5 cm; peak to peak voltage, measured in water, about 1.4–2.5 V[11]; duration of fish pulse 300 µsec) were held each for several days in 150 l glass aquarium at 26–27.5°C, into which *Mormyrus rume* (20.5 cm; 6.0 V[11]; 750 µsec) was introduced each time for 3–5 min. The attacks were filmed on a video equipment (SONY). A system of 3 pairs of carbon electrodes, oriented vertically and horizontally, was used to pick up all electrical pulses of the fish[12]. Each pair of the 3 pairs of carbon electrodes was connected to one preamplifier. Amplified pulses were rectified, the three signals summarized and fed into a tape recorder. To distinguish the discharges emitted by the 2 fish – characterized by different amplitude and duration – the tape was played back into an oscilloscope. The images on the screen were recorded on 35 mm film moving past the open shutter at 50 cm/sec. Tape recordings were also fed into a computer (Didac 800) which had been programmed to perform intervalhistograms and instantaneous frequency histograms. Each experiment was started with control recordings of each fish prior to the attacking behaviour. Results obtained in one fish (*G. petersii* No. 1) are described in detail.

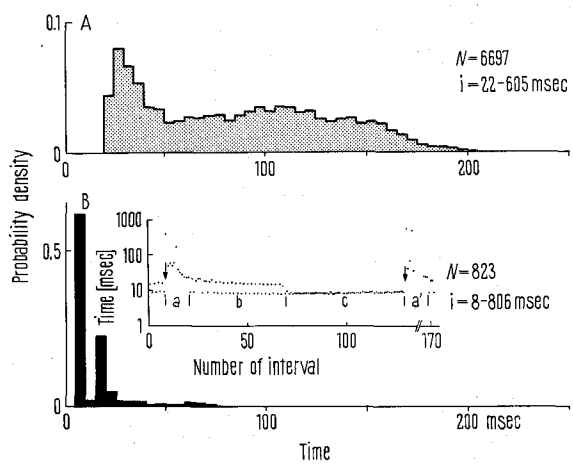

Fig. 1. Interval histograms of electrical pulses of *G. petersii* No. 1. A) control, $T = 10$ min. B) aggressive behaviour, $T = 20$ sec. *N*, number of intervals; i, shortest and longest interval. Probability density of intervals plotted against intervals duration.
inset in B). 'High frequency' bursts of *G. petersii* No. 1 during aggressive behaviour. Interval (ms) plotted against number of interval. a) and a') Inter-burst activity. b) and c) Burst with 2 types of activity (see text). Duration of b) 535 msec, of c) 692 msec. Arrows, end of bursts.

During 10 min control *G. petersii* No. 1 discharged a mean frequency of 11 Hz. In Figure A a characteristic peak on the left side of the histogram of pulse intervals corresponds to spontaneous burst-like acceleration of the discharge frequency (mode: 26 ms $\hat{=}$ 38 Hz). The shortest interval observed during the entire control period was of 22 ms.

During the following 180 sec period of aggressive behaviour, the discharge pattern of *G. petersii* No. 1 changed characteristically. The mean frequency now increased to 41 Hz and *G. petersii* emitted long lasting 'high frequency' bursts (inset in Figure B). In each of the four 180 sec periods of experiment 38–47 'high frequency' bursts occurred. The bursts displayed 2 kinds of pattern: in one the low frequency interburst activity (a in inset of Figure B) was followed by a particular pattern, in which intervals of 8 and 15 msec regularly alternated; (b in inset of Figure B), the final part of these bursts was characterized by a constant frequency of 117 Hz; (c in inset of Figure B). The second kind of pattern was similar to the part b) of the first kind of burst (see inset in Figure B, end of preceeding burst before a). In both cases the bursts ended abruptly (arrows in inset of Figure B), each one being separated from the other by an interburst activity characterized by several long intervals up to 806 msec.

1 H. W. Lissmann, J. exp. Biol. *35*, 156 (1958).
2 F. P Möhres, Naturwissenschaften *44*, 341 (1957).
3 W. Harder, A. Schief and H. Uhlemann, Z. vergl. Physiol. *48*, 302 (1964).
4 K. Sänger, Experientia *23*, 868 (1967).
5 P. Moller and R. Bauer, in preparation.
6 F. J. Mandriota and R. L. Thompson, Science *150*, 1740 (1965).
7 W. Harder, A. Schief and H. Uhlemann, Z. vergl. Physiol. *54*, 89–108 (1967).
8 P. Moller, Anim. Behav. *18*, 768 (1970).
9 F. P. Möhres, Natur Volk *91*, 1 (1961).
10 P. Black-Cleworth, Anim. Behav. Monogr. *3*, 1 (1970).
11 Resistivity of water 0.92 KOhms/cm.
12 R. Bauer, J. Physiol., Paris *62*, suppl. 3, 341 (1970).